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EFFECTS OF pH ON THE MYOFILAMENTS AND THE SARCOPLASMIC RETICULUM OF SKINNED CELLS FROM CARDIAC AND SKELETAL MUSCLES

BY ALEXANDRE FABIATO AND FRANÇOISE FABIATO
From the Department of Physiology, Medical College of Virginia, Richmond, Virginia 23298, U.S.A.

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SUMMARY

1. The effects of decreasing pH from 7·40 to 6·20 on the tension developed by direct activation of the myofilaments and by Ca^{2+} release from the sarcoplasmic reticulum were studied comparatively in segments of single cells of skeletal muscle (frog semitendinosus) and cardiac muscle (rat ventricle) from which the sarcolemma had been removed by micro-dissection (skinned muscle cells).

2. The concentration of free Ca^{2+} in the solutions was buffered with ethylene glycol-bis (β-aminoethylether *N,N'*-tetraacetic acid (EGTA). The change of the buffer capacity of a given [total EGTA] caused by varying pH and the uncertainty about the value of the equilibrium constant for Ca-EGTA have been taken into account in the interpretation of the results.

3. Decreasing pH from 7·40 to 6·20 produced an increase in the [free Ca^{2+}] required for the myofilaments to develop 50 % of the maximum tension by a factor of about 5 in skinned cardiac cells but of only 3 in skeletal muscle fibres. In addition, acidosis depressed the maximum tension developed in the presence of a saturating [free Ca^{2+}] by approximately the same amount in the two tissues.

4. The pH optimum for loading the sarcoplasmic reticulum of skinned fibres from skeletal muscle decreased when the pCa ($-\log$ [free Ca^{2+}]) in the loading solution decreased. The optimum was pH 7·40–7·00 for a loading at pCa 7·75, pH 7·00–6·60 at pCa 7·00 and pH 6·60–6·20 at pCa 6·00.

5. The pH optimum for loading the sarcoplasmic reticulum of skinned cardiac cells with a solution at pCa 7·75 was about pH 7·40 as in skeletal muscle fibres. But the cardiac sarcoplasmic reticulum could not be loaded with a [free Ca^{2+}] much higher than pCa 7·75 because a higher [free Ca^{2+}] triggered a Ca^{2+}-induced release of Ca^{2+} from the sarcoplasmic reticulum.

6. The pH optimum of about 7·40 for the loading of the cardiac sarcoplasmic reticulum was also optimum for the Ca^{2+}-induced release of Ca^{2+} from it.

7. It was concluded that the effects of acidosis on the cardiac sarcoplasmic reticulum accentuate the depressive action of decreasing pH on the myofilaments. This may explain the pronounced depression of contractility observed during acidosis in cardiac muscle. In contrast, a moderate acidosis causes an effect on skeletal muscle sarcoplasmic reticulum that could compensate for the depressive action on the myofilaments, which is, in addition, less pronounced than in cardiac muscle.

INTRODUCTION

Since the original observations by Gaskell (1880), both the negative inotropic effect of acidosis and the positive inotropic effect of alkalosis on heart muscle have been well documented. These observations gained further clinical interest when Katz & Hecht (1969) suggested that intracellular acidosis could be a cause for the decrease of contractility observed during myocardial ischaemia.

A largely unexplained finding is that the inotropic effects of varying pH on skeletal muscle are much less pronounced than those observed in cardiac muscle (Pannier & Leusen, 1968; Pannier, Weyne & Leusen, 1970). Part of the negative inotropic effect of acidosis in cardiac muscle could be caused by a depression due to acidosis of the Ca^{2+} current across the sarcolemma (Chesnais, Coraboeuf, Sauviat & Vassas, 1975; Kolhardt, Haap & Figulla, 1976), whereas no significant trans-sarcolemmal Ca^{2+} current participates in the activation of the intact skeletal muscle (Endo, 1977).

However, this effect of acidosis on the surface membrane is unlikely to represent a major mechanism of its negative inotropic action because the metabolic acidosis caused by large anions has much less effect than the respiratory acidosis caused by CO_2, which diffuses easily into the cell. Thus, an increase of P_{CO_2} that is insufficient to produce any detectable effect on the plateau of the cardiac action potential causes a considerable decrease of the force developed by the intact cardiac muscle (Poole-Wilson & Langer, 1975). This suggests that the mechanism of the negative inotropic effect of acidosis and the cause of the differences between cardiac and skeletal muscles may be intracellular. A given increase of P_{CO_2} should cause at least as large a variation of intracellular pH in skeletal muscle as in cardiac muscle since the intact skeletal muscle has a smaller buffer capacity than cardiac muscle for variations of intracellular pH caused by CO_2 (Clancy & Brown, 1966). Thus, it seems that the difference between skeletal and cardiac muscles with respect to the action of intracellular acidosis should be explained by unequal effects on the organelles that control contraction and excitation–contraction coupling: the myofilaments and the sarcoplasmic reticulum.

To compare the effects of acidosis and alkalosis on the myofilaments and the sarcoplasmic reticulum of cardiac and skeletal muscles, we have studied the effects of varying pH on the tension developed by segments of single cells of cardiac and skeletal muscles from which the sarcolemma had been removed by micro-dissection (skinned muscle cells). These preparations permitted the control of the pH and of the concentration of free Ca^{2+} ions (i.e. [free Ca^{2+}]) in the medium bathing the myofilaments and the sarcoplasmic reticulum. The study was done comparatively in skinned skeletal muscle fibres from the frog semitendinosus and skinned cardiac cells from the rat ventricle because most of the previous data on skinned muscle cells were obtained from these two preparations.

Recently, Tsien (1976) reviewed the data from the literature on the effects of acidosis on cardiac muscle contraction and mentioned some of our preliminary observations on this subject. These preliminary observations confirmed the finding by Schädler (1967) that acidosis depresses the submaximum Ca^{2+}-activated tension but failed to show a significant decrease of the maximum tension produced by an

optimum [free Ca^{2+}] when pH was decreased from 7·40 to 6·60 in skinned cardiac cells from the rat ventricle. In contrast, Schädler (1967), who used much broader variations of pH, observed that acidosis decreased the maximum tension. Accordingly, the pH range was extended in the present study to pH 6·20, which seems to be the lowest value for which EGTA has a Ca^{2+} buffer capacity sufficient to permit the data to be interpretable. With respect to the effects of pH on the sarcoplasmic reticulum particular attention was placed on testing the physiological significance of the observation by Nakamaru & Schwartz (1970, 1972) that acidosis increases and alkalosis decreases the Ca^{2+} uptake by the fragmented sarcoplasmic reticulum of cardiac and skeletal muscles.

This study proposes an explanation for the more pronounced depression of cardiac than skeletal muscle contraction observed during acidosis on the basis of a more alkaline optimum pH for Ca^{2+} loading of the sarcoplasmic reticulum and of a larger depression of the contraction of the myofilaments in cardiac than in skeletal muscle. Reaching this conclusion required a critical study of the influence of Ca^{2+} binding by ethyleneglycol-bis (β-aminoethylether) *N,N'*-tetraacetic acid (EGTA) on the interpretation of the data.

METHODS

Solutions

The method of successive approximations described by Botts, Chashin & Schmidt (1966) was used to establish a computer program for calculating the total concentrations of the electrolytes and substrates necessary to make solutions at specified free ion concentrations. This program permits the use of an unlimited number of metals and ligands and can be operated either by entering the concentrations of free ions desired, and obtaining as the output the total concentrations of electrolytes and substrates necessary to make the solutions or by entering the total concentrations and obtaining as the output the concentrations of free ions.

The following absolute equilibrium constants were used: $[HATP^{3-}]/[H^+][ATP^{4-}] = 8{\cdot}91 \times 10^6\ M^{-1}$ (Smith & Alberty, 1956); $[H_2ATP^{2-}]/[H^+][HATP^{3-}] = 1{\cdot}12 \times 10^4\ M^{-1}$ (Martell & Schwarzenbach, 1956); $[CaATP^{2-}]/[Ca^{2+}][ATP^{4-}] = 9{\cdot}32 \times 10^3\ M^{-1}$ and $[CaHATP^-]/[Ca^{2+}][HATP^{3-}] = 6{\cdot}30 \times 10^1\ M^{-1}$ (Nanninga, 1961); $[MgATP^{2-}]/[Mg^{2+}][ATP^{4-}] = 2{\cdot}14 \times 10^4\ M^{-1}$ and $[MgHATP^{-2}]/[Mg^{2+}][HATP^{3-}] = 2{\cdot}09 \times 10^3\ M^{-1}$ (Nanninga, 1961); $[HEGTA^{3-}]/[H^+][EGTA^{4-}] = 2{\cdot}88 \times 10^9\ M^{-1}$, $[H_2EGTA^{2-}]/[H^+][HEGTA^{3-}] = 7{\cdot}08 \times 10^8\ M^{-1}$, $[H_3EGTA^-]/[H^+][H_2EGTA^{2-}] = 4{\cdot}79 \times 10^2\ M^{-1}$ and $[H_4EGTA]/[H^+][H_3EGTA^{3-}] = 10^2\ M^{-1}$ (Schwarzenbach, Senn & Anderegg, 1957); $[MgEGTA^{2-}]/[Mg^{2+}][EGTA^{4-}] = 1{\cdot}62 \times 10^5\ M^{-1}$ and $[MgHEGTA^-]/[Mg^{2+}][HEGTA^{3-}] = 2{\cdot}34 \times 10^3\ M^{-1}$ (Schwarzenbach & Senn, 1964); $[KATP^{3-}]/[K^+][ATP^{4-}] = 8\ M^{-1}$ (Botts *et al.* 1966).

The most widely used absolute association constants for the formation of Ca-EGTA complexes are those calculated by Schwarzenbach *et al.* (1957) from data obtained with a pH titration method in the presence of 0·1 M-KCl: $[CaEGTA^{2-}]/[Ca^{2+}][EGTA^{4-}] = 1{\cdot}00 \times 10^{11}\ M^{-1}$ and $[CaHEGTA^-]/[Ca^{2+}][HEGTA^{3-}] = 2{\cdot}14 \times 10^5\ M^{-1}$. These values were used by Schädler (1967), Bozler (1968), Portzehl, Zaoralek & Gaudin (1969) and Williams, Collins, Muir & Stephens (1975) to study the effects of pH on the tension developed by glycerinated preparations from skeletal and cardiac muscles and on the ATPase activity of the myofibrils. These values were also used for preliminary data on the effects of pH on skinned skeletal muscle fibres (Ashley & Moisescu, 1974). In addition, equilibrium constants for Ca-EGTA close to those reported by Schwarzenbach *et al.* (1957) were found by Moisescu (1976), who used a pH metric method, and by Dipolo, Requena, Brinley, Mullins, Scarpa & Tiffert (1976), who used the metalochromatic dye arsenazo III to measure the [free Ca^{2+}] remaining after the binding of Ca^{2+} to EGTA.

From data obtained with a Chelex partition method, Briggs & Fleishman (1965) calculated a much lower absolute stability constant of $2{\cdot}14 \times 10^{10}\ M^{-1}$ for the formation of $CaEGTA^{2-}$ from Ca^{2+} and $EGTA^{4-}$. However, this value must be corrected because of the presence of oxalate in the solution.

Ogawa (1968) measured the [free Ca^{2+}] remaining after the binding of Ca^{2+} to EGTA by

recording with dual wave-length spectrophotometry the change in light absorption that occurs when Ca^{2+} complexes with murexide. Ogawa made the measurement in the presence of 0·1M KCl and with various pH buffers. When 20 mM Tris maleate was used as a pH buffer, the calculated absolute equilibrium constants were $[CaEGTA^{2-}]/[Ca^{2+}][EGTA^{4-}] = 2{\cdot}25 \times 10^{10}\ M^{-1}$ and $[CaHEGTA^{-}]/[Ca^{2+}][HEGTA^{3-}] = 2{\cdot}10 \times 10^{5}\ M^{-1}$. These equilibrium constants calculated by Ogawa (1968) were used by Chen-Liu & Endo (1973) for a preliminary study of the effects of pH on the tension developed by Ca^{2+}-activated skinned fibres from the frog semitendinosus.

Godt & Briggs (quoted by Godt, 1974) calculated from data obtained with a technique of Chelex partition an equilibrium constant which is very close to that obtained by Ogawa (1968): $[CaEGTA^{2-}]/[Ca^{2+}][EGTA^{4-}] = 2{\cdot}62 \times 10^{10}\ M^{-1}$. This constant was used by Robertson & Kerrick (1976) for preliminary data on the effects of pH on the tension developed by Ca^{2+}-activated skinned fibres from the frog semitendinosus. Thus, the discrepancy between the preliminary results by Chen-Liu & Endo (1973) and by Robertson & Kerrick (1976) cannot be explained by a difference in the equilibrium constants for Ca-EGTA.

Recently, Allen, Blinks & Prendergast (1977) used the bioluminescence of aequorin to measure the [free Ca^{2+}] remaining after the binding of Ca^{2+} to EGTA. The experimental conditions used by Allen *et al.* (1977) were much closer to those used in the present study than were those used for previous determinations of the equilibrium constant of Ca-EGTA. In one determination made at pH 7·00 and at 21 °C in the presence of 5mM piperazine-*N*,*N*′-bis(2-ethanesulphonic acid) (PIPES, pH buffer with a pK of 6·70 at 37 °C and 6·80 at 20 °C) and 150 mM KCl, Allen *et al.* found a logarithm to base 10 of the apparent stability constant for Ca-EGTA of 6·45. In six unpublished observations done under the same conditions by D. G. Allen & J. R. Blinks (personal communication, 1977), the log of the apparent stability constant was 6·400 ± 0·018 (S.E. of the mean). Using this apparent stability constant and the absolute stability constants for the formation of complexes between H^+ and EGTA of Schwarzenbach *et al.* (1957), an absolute equilibrium constant intermediary between those calculated by Schwarzenbach *et al.* (1957) and Ogawa (1968) is obtained: $[CaEGTA^{2-}]/[Ca^{2+}][EGTA^{4-}] = 5{\cdot}20 \times 10^{10}\ M^{-1}$. The apparent stability constants at different pH values calculated from this absolute equilibrium constant agree well with the preliminary measurements made by D. G. Allen & J. R. Blinks (unpublished) with aequorin at pH 6·60 and 7·40.

In summary, the stability constants proposed for the Ca-EGTA complex can be separated into three groups: (1) those close to the stability constant proposed by Schwarzenbach *et al.* (1957); (2) those close to the stability constant by Ogawa (1968); and (3) the most recent stability by Allen *et al.* (1977). For the present study, the stability constants for the different equilibriums listed previously, including the constant for Ca-EGTA defined by Schwarzenbach *et al.* (1957), were entered into the computer program. The computer calculated the total concentrations of electrolytes and substrates necessary to obtain specified values of pCa ($-\log$ [free Ca^{2+}]) in the presence of a constant pMgATP ($-\log$ [$MgATP^{2-}$]) of 2·50 and a constant pMg ($-\log$ [free Mg^{2+}]) of 3·50. The total concentrations (i.e. the output of the computer) were automatically re-entered with one of the two other stability constants for Ca-EGTA (stability constant by Allen *et al.* 1977, and during a subsequent run, with a stability constant by Ogawa, 1968). Then the computer reversed the initial process and calculated the free ion concentrations. By this procedure, the correspondence between the pCa obtained with the same total concentrations of electrolytes and substrates but with three different stability constants for Ca-EGTA was obtained. To permit easy comparison between our physiological data and those reported by others using different stability constants, the correspondence of the values of pCa calculated with the different equilibrium constants for Ca-EGTA is shown in Table 1. This table was limited, however, to the values of pCa used in the present study.

The results of the computations indicated that, under the conditions used in this study, changing the equilibrium constant for Ca-EGTA did not modify the pMgATP. In contrast, the pMg was slightly modified. However, the largest modification was pMg 3·46 (instead of 3·50) at pH 6·20 and pCa 3·74 when the constant by Ogawa (1968) was substituted for the constant by Schwarzenbach *et al.* (1957). Such a small change of pMg has no measurable effect on tension (Fabiato & Fabiato, 1975*b*). Accordingly, the values of pMg are not indicated in Table 1. This table can be used to replot the data presented in this article, which are expressed using the stability constant by Schwarzenbach *et al.* (1957) for the Ca-EGTA complex without any implication that this stability constant is more valid than the others.

The pH was measured with an accuracy of ± 0·01 unit with a Corning model 112 digital pH

TABLE 1. Correlations of the pCa values calculated with different absolute stability constants for the Ca-EGTA complex. In all cases, [total EGTA] was 4·0 mM, pMgATP was 2·50 and pMg was between 3·46 and 3·50

pH	pCa Schwarzenbach *et al.* (1957)	pCa Allen *et al.* (1977)	pCa Ogawa (1968)
7·40	9·00	8·71	8·35
7·40	7·75	7·46	7·10
7·40	7·50	7·21	6·85
7·40	7·25	6·96	6·60
7·40	7·00	6·71	6·35
7·40	6·75	6·46	6·10
7·40	6·50	6·21	5·86
7·40	6·25	5·97	5·62
7·40	6·00	5·73	5·40
7·40	5·75	5·50	5·21
7·40	5·50	5·30	5·07
7·40	5·00	4·93	4·82
7·40	4·50	4·49	4·46
7·40	4·00	4·00	3·99
7·00	9·00	8·71	8·35
7·00	7·75	7·46	7·10
7·00	7·00	6·71	6·35
7·00	6·75	6·46	6·10
7·00	6·50	6·21	5·85
7·00	6·25	5·96	5·61
7·00	6·00	5·71	5·36
7·00	5·75	5·47	5·13
7·00	5·50	5·23	4·93
7·00	5·00	4·83	4·63
7·00	4·50	4·45	4·36
7·00	4·00	3·99	3·97
6·60	9·00	8·72	8·35
6·60	7·75	7·47	7·10
6·60	7·00	6·72	6·35
6·60	6·50	6·22	5·85
6·60	6·25	5·97	5·60
6·60	6·00	5·72	5·35
6·60	5·75	5·47	5·11
6·60	5·50	5·22	4·87
6·60	5·00	4·76	4·46
6·60	4·50	4·36	4·17
6·60	4·00	3·96	3·89
6·20	9·00	8·72	8·37
6·20	7·75	7·47	7·12
6·20	7·00	6·72	6·37
6·20	6·50	6·22	5·87
6·20	6·25	5·97	5·62
6·20	6·00	5·72	5·38
6·20	5·75	5·47	5·13
6·20	5·50	5·22	4·89
6·20	5·00	4·73	4·42
6·20	4·50	4·28	4·03
6·20	4·00	3·89	3·74

meter (Corning Glass Works, Medfield, Mass., U.S.A.). The pH was adjusted in the solution at 22 °C and during the experiment the temperature was maintained at 22 ± 0·1 °C by the temperature-controlled stage of the microscope. Imidazole (pK 6·90 at 22 °C, obtained from Sigma Chemical Co., St Louis, Mo., U.S.A.) was used as a pH buffer at the four values of pH studied: 7·40, 7·00, 6·60 and 6·20. The concentration of imidazole was 20 mM in all solutions. Ogawa (1968) has reported that the apparent stability constant for Ca-EGTA varied with the concentration of imidazole, but only when this concentration was less than 20 mM. Furthermore, D. C. Allen & J. R. Blinks (unpublished) found that the log of their apparent stability constant was 6·42 in the presence of 5 mM imidazole and 6·37 in the presence of 20 mM imidazole. These results of single determinations are very close to the mean value of 6·40 that was observed in the presence of 5 mM PIPES.

Additional experiments at pH 7·40 were done with tris(hydroxymethyl)aminomethane (Tris, pK 7·90 at 37 °C, obtained from Sigma), whereas some experiments at pH 6·60 and 6·20 were done with (bis(2-hydroxyethyl)amino)-tris(hydroxymethyl)methane (Bis-Tris, pK 6·46 at 37 °C, obtained from Sigma). The change of the pH buffer did not modify the tension developed by a given cell at a given pH and pCa. Accordingly, the results are presented without a distinction between the pH buffers used.

All solutions contained 7 mM glucose. Unless otherwise specified, the pMgATP was 2·50 and the pMg was 3·50. For each solution, the computer output gave the concentration of KCl to be added to obtain an ionic strength of 0·160 M. Godt & Briggs (quoted by Godt, 1974) reported that variations of [KCl] did not affect significantly the apparent stability constant for the Ca-EGTA complex. In contrast, Dipolo *et al.* (1976) and Allen & Blinks (unpublished) observed that increasing [KCl] decreased the apparent stability constant of the Ca-EGTA complex. But this effect was apparently caused by varying the ionic strength of the solution rather than by a binding of K^+ ions to EGTA. Yet these observations show that the use of stability constants determined at ionic strengths different from those used for the physiological experiments may introduce an error. Finally, the total Ca concentration in the solutions was controlled by atomic absorption spectrophotometry.

Preparation of skinned muscle cells and tension recording

The techniques for the preparation of skinned cardiac cells of rat ventricle and for tension recording were similar to those described previously (Fabiato & Fabiato, 1975*a*). The segments of skinned cardiac cells were 8–12 μm wide and 30–60 μm long. Small bundles of myofibrils of frog semitendinosus 8–12 μm wide and 30–60 μm long were prepared with exactly the same technique as that used for the cardiac muscle. In all experiments, the cells were stretched to a resting sarcomere length of 2·20–2·30 μm, which corresponds to the maximum active tension. The length and the width of the preparations, which are indicated in the legends of the figures, were measured after the cells had been set at this sarcomere length of 2·20–2·30 μm.

Results were given as mean ± standard deviation (S.D.), compared by paired *t* tests and regarded as significantly different when $P < 0·05$. The S.D. was used instead of the more commonly employed S.E. of the mean simply because it permitted a more accurate graphic representation.

RESULTS

Effects of pH on the Ca^{2+}-activated tension developed by the myofilaments

The effects of varying pH on the relation between [free Ca^{2+}] and the tension developed by the myofilaments were studied in the presence of 4 mM total EGTA, which was generally sufficient to buffer any variation of [total Ca] caused by the Ca^{2+} sequestration by and release from the sarcoplasmic reticulum. This has been verified by control experiments on skinned skeletal muscle fibres from the frog semitendinosus in which 10 mM total EGTA was used. The level of the plateau of tension was not significantly different at any given pCa and pH when either 4 or 10 mM total EGTA was used. However, the rate of tension development was slower in the presence of 4 mM than in the presence of 10 mM total EGTA at pH 6·20 and [free Ca^{2+}] lower than pCa 6·00.

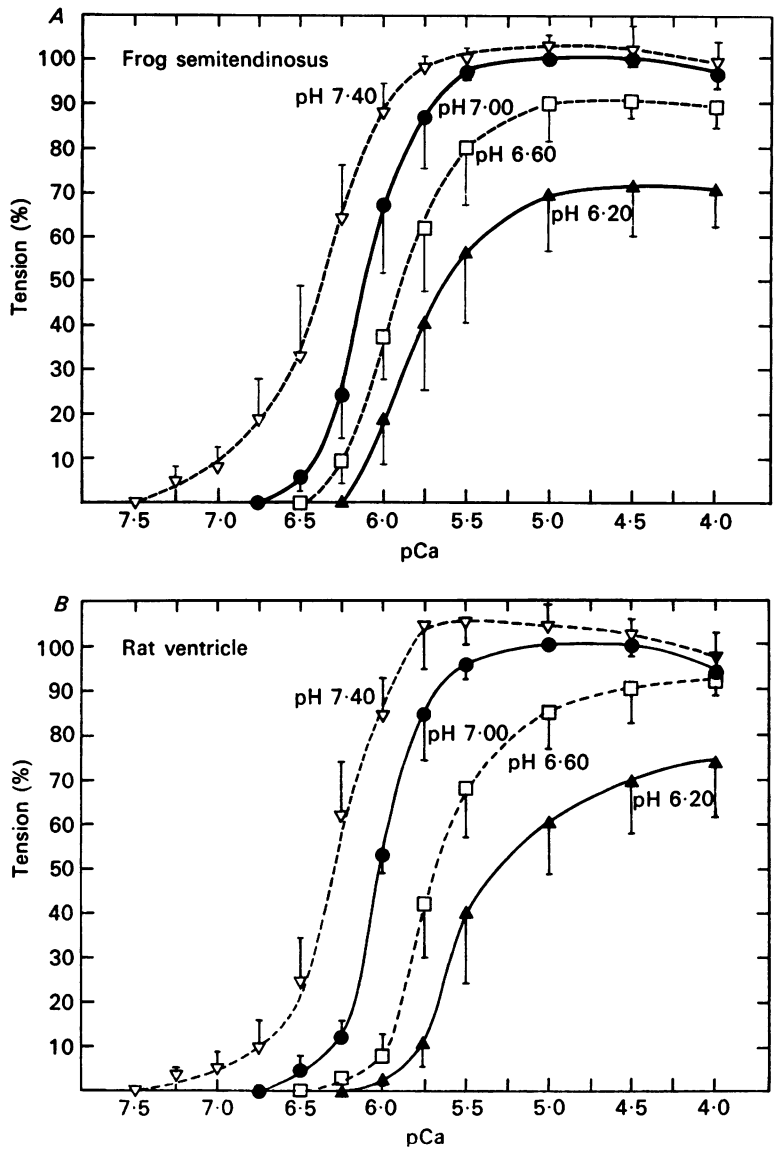

Fig. 1. Effects of varying pCa and pH on the tension developed by segments of skinned skeletal (*A*) and cardiac (*B*) muscle cells in the presence of 4·0 mM total EGTA, pMg 3·50 and pMgATP 2·50. The stability constant by Schwarzenbach *et al.* (1957) was used for the Ca-EGTA complex. The tension developed by each segment of fibre at a given pCa and a given pH was expressed as a percentage of the tension developed by the same segment at pCa 5·00 and pH 7·00. Each point corresponds to the mean of ten to twenty determinations except for the points indicating pCa 6·00 and pH 7·00, where fifty determinations were used. Each vertical bar corresponds to one S.D., which is shown in only one direction for clarity. The decrease of tension for supra-optimal [free Ca^{2+}] shown in skinned cardiac cells has already been reported for the myofibrillar ATPase activity (Portzehl *et al.* 1969).

The two protocols used to compare the tonic tension developed by skinned muscle cells at various pH and pCa values were the same as previously used for the study of the effects of Mg^{2+} (Fabiato & Fabiato, 1975*b*). The tension obtained from a given cell at a given pH and pCa was expressed as a percentage of the tension developed by the same cell at pCa 5·00 and pH 7·00 (Fig. 1). This point was chosen as the reference although it did not always correspond to the maximum tension developed by the cell. All the data were obtained at pMg 3·50 and pMgATP 2·50.

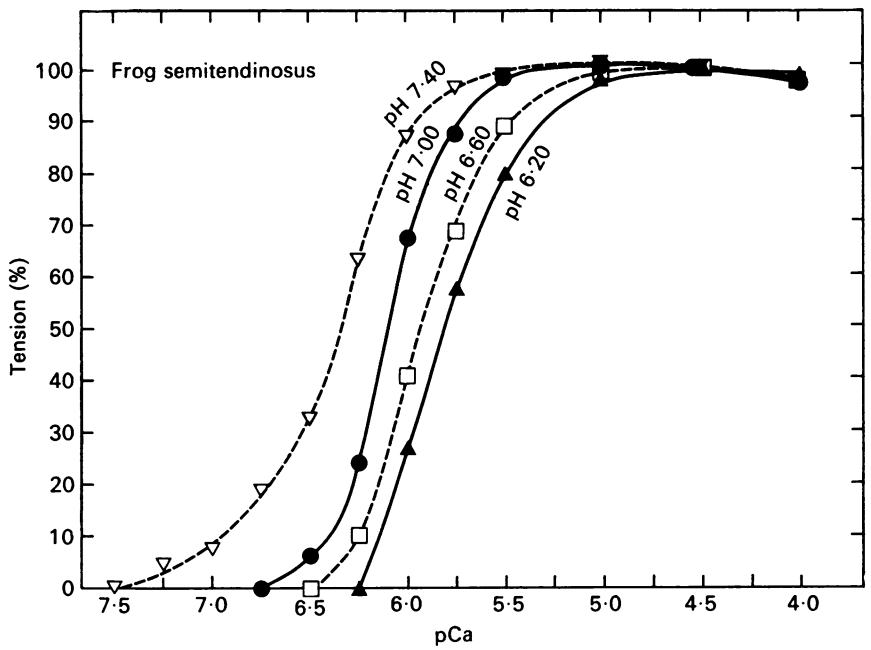

Fig. 2. Normalization of the data of Fig. 1*A* by expressing the tension developed by each segment of fibre at a given pCa and a given pH as a percentage of the maximum tension developed by the same segment at this pH.

Decreasing pH from 7·40 to 6·20 resulted in three modifications: (1) an increase in the threshold of [free Ca^{2+}] required for contraction, (2) a shift to the right of the force–pCa curve so that a higher [free Ca^{2+}] was required to obtain the same tension when the pH was lower and (3) a decrease of the maximum tension obtained in the presence of a high (optimal) [free Ca^{2+}] (Fig. 1).

The effects of decreasing pH on the [free Ca^{2+}] threshold for contraction and on the submaximal tension developed at [free Ca^{2+}] lower than pCa 6·00 were more pronounced when the pH varied by the same fraction of a pH unit but in the alkaline range (pH 7·40–7·00) rather than in the acidic range (pH 6·60–6·20). A replot of the data, expressing the tension obtained at different pCa values but at a given pH as a function of the maximum tension developed at this pH, still showed this non-proportional shift (Fig. 2). Changing the absolute stability constant for Ca-EGTA would change the shape of the curves but the effect of decreasing pH on submaximal tension would still appear smaller in the acidic rather than in the alkaline range.

Therefore, these data do not support the hypothesis of a simple competition between H^+ and Ca^{2+} for a single class of binding sites on troponin.

The effect of decreasing pH on the submaximal tension was more pronounced in skinned cardiac cells than in skeletal muscle fibres (Fig. 1). When pH was decreased from 7·40 to 6·20, the [free Ca^{2+}] required to obtain 50% of maximum tension increased by a factor of 3·47 (from pCa 6·33 to 5·79) in skeletal muscle as compared to a factor of 5·50 (from pCa 6·30 to 5·56) in cardiac muscle. Should the stability constant for Ca-EGTA described by Ogawa (1968) be used, the factor of increase would be 3·39 in skeletal muscle and 5·25 in cardiac muscle.

In contrast to the submaximum tension, the maximum tension was more depressed when the pH was decreased in the acidic than in the alkaline range (Fig. 1). A decrease of pH from 6·60 to 6·20 produced a large and highly significant ($P < 0·001$) decrease of maximum tension in both preparations. Decrease of pH from 7·00 to 6·60 produced a decrease of the tension developed at pCa 4·00 that was significant in skeletal muscle but not in cardiac muscle. Finally, the decrease of maximum tension produced by decreasing pH from 7·40 to 7·00 was not significant in either tissue.

The effect of acidosis on the maximum tension was completely reversible by returning to neutral pH, but was not overcome by increasing the [free Ca^{2+}]. Therefore, this effect cannot be caused by a direct competition between Ca^{2+} and H^+ for a binding site on troponin. It could be explained by an influence of H^+ ions on any of the steps of the contractile processes that occur after the interaction between Ca^{2+} and troponin. A decrease of pH could decrease the affinity between the subunits of troponin or modify the conformation of tropomyosin. Alternatively, or additionally, acidosis could limit the force developed by the interaction between actin and myosin (decrease of force developed per cross-bridge or of number of cross-bridges formed) in the presence of an optimal [free Ca^{2+}].

This hypothesis of a modification of the contractile proteins induced by varying pH and independent of any effect on the Ca^{2+} regulation has been tested by studying the effects of varying pH on the rigor tension developed in the absence of Ca^{2+}. Previous studies have shown that at pH 7·00, decreasing the [$MgATP^{2-}$] in the virtual absence of Ca^{2+} (pCa 9·50) resulted in the development of a tonic tension. The maximum rigor tension was observed at pMgATP 5·50 in skinned cells from both the rat ventricle and the frog semitendinosus (Fabiato & Fabiato, 1975*b*). Decreasing pH from 7·00 to 6·20 decreased this maximum rigor tension by $33·22 \pm 5·41$% (S.D. of eleven observations) in the frog semitendinosus and $28·71 \pm 6·22$% (S.D. of fourteen observations) in the rat ventricle.

Effects of pH on the Ca^{2+} loading of and Ca^{2+} release from the sarcoplasmic reticulum

The method used for quantitative comparison of the effect of varying pH on the Ca^{2+} loading of the sarcoplasmic reticulum of cardiac and skeletal muscles was a modification of that developed by Endo (1975, 1977). This method consisted of making all the changes of pCa and pH capable of loading the sarcoplasmic reticulum or of releasing Ca^{2+} from the reticulum in the presence of a [total EGTA] high enough to prevent any amount of total Ca released from inducing a contraction. After these interventions modifying the Ca^{2+} content of the sarcoplasmic reticulum the

preparation was washed with a relaxing solution (pCa 9·00) at a constant pH of 7·00. The experiment ended with a perfusion by a solution containing 10 mM caffeine at a constant pH of 7·00, a pCa of 8·30 and a [total EGTA] of only 0·20 mM. The amplitude of the phasic contraction induced by caffeine gave an estimate of the amount of Ca^{2+} stored in the sarcoplasmic reticulum after the modifications of pH and pCa made in the presence of a high [total EGTA]. Thus, a larger caffeine-induced contraction indicated that more Ca^{2+} had been stored in the sarcoplasmic reticulum during the preceding modifications of pCa and pH.

The [total EGTA] used during the caffeine-induced contraction was much lower than that used by Endo (1975, 1977), because caffeine released much less Ca^{2+} in these small preparations studied at 22 °C than in the large preparations used at 0 °C by Endo. This lesser amount of Ca^{2+} released and the use of imidazole (which is a much stronger pH buffer at pH 7·00 than the Tris that was used by Endo at pH 6·80) rendered negligible in our experiments the variation of pH caused during caffeine-induced release of Ca^{2+} by the reaction: $Ca^{2+} + H_2EGTA^{2-} \rightarrow CaEGTA^{2-} + 2H^+$ (Moisescu, 1976). The duration of the phasic contraction induced by caffeine under constant conditions was extremely variable. However, the spontaneous relaxation always began less than 5 sec after the beginning of the caffeine perfusion. Accordingly, the perfusion was limited to 5 sec to avoid a progressive damage of the preparation. The amplitude of the caffeine-induced contraction (rather than the area below the curve as used by Endo, 1975, 1977) was used for quantitative analysis. The reproducibility of the amplitude of the contraction obtained with 10 mM caffeine after loading at a given pCa and pH confirmed the observation by Endo (1975, 1977) that caffeine releases a relatively constant fraction of the Ca^{2+} contained by the sarcoplasmic reticulum even if it does not empty the sarcoplasmic reticulum completely (Fabiato & Fabiato, 1977).

The term 'loading' used in the presentation of the results does not imply which compartment of the sarcoplasmic reticulum is loaded: Ca^{2+} could be bound or stored inside, outside or within the sarcoplasmic reticulum membrane. The relation between the [free Ca^{2+}] in the bathing medium and the [free Ca^{2+}] inside the lumen of the sarcoplasmic reticulum is not known.

Fig. 3 shows the results obtained with this method on a skinned skeletal muscle fibre. As in all experiments, pMg was constant at 3·50 and pMgATP at 2·50. The sarcoplasmic reticulum was loaded during 2–3 min by a perfusion solution containing 4·0 mM total EGTA with a constant pCa of 7·00 but at various pH values. This loading perfusion was followed by a 2 sec washing and a caffeine perfusion. Only the amplitude of the caffeine-induced contraction provided information on the Ca^{2+} content of the sarcoplasmic reticulum. Any tension change observed during the loading perfusion (Fig. 3*A* and *F*) was explained by a direct effect of the perfusion medium on the myofilaments because this medium contained a [total EGTA] sufficient to buffer any amount of Ca^{2+} released from the sarcoplasmic reticulum. The experiments at different pH values were done in an equal number of series of increasing or decreasing pH. For statistical analysis, the amplitude of the caffeine-induced contraction developed by a given cell was normalized by expressing it as a percentage of the amplitude of the tonic tension developed by the same cell at pCa 5·00 and pH 7·00 in the presence of 4·0 mM total EGTA. The maximum amplitude of the contraction induced by caffeine was observed after a loading with a perfusion at a pH optimum that varied between 7·00 and 6·60. The amplitude of the contraction decreased significantly when the pH of the loading solution was either decreased to 6·20 or increased to 7·40. Yet the amplitude of the caffeine-induced contraction was much smaller ($P < 0·001$) after loading at pH 6·20 than at pH 7·40 (Table 2).

This experiment was also done with variations of the [free Ca^{2+}] of the solution used

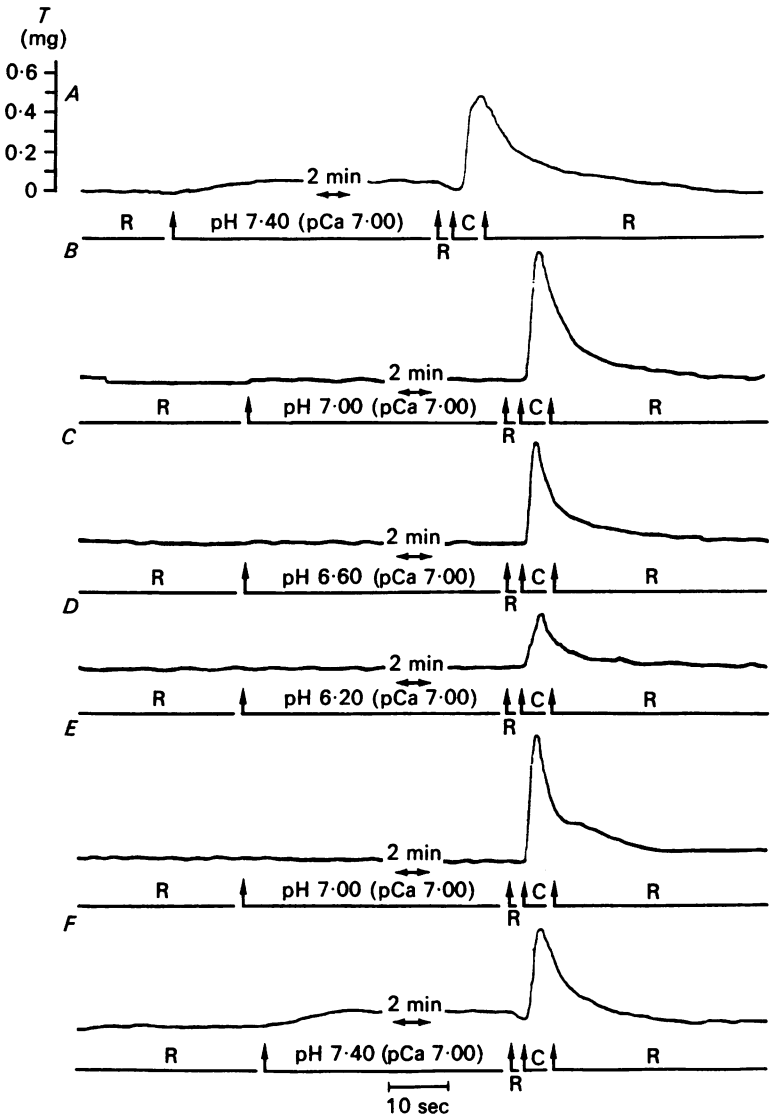

Fig. 3. Effects of changing the pH of the Ca^{2+}-loading solution on the amplitude of the contraction induced by caffeine at pH 7·00 in a 10 μm wide and 48 μm long segment of skinned fibre from the frog semitendinosus. The experiments were done in the order in which they are shown. Between the recordings shown in two consecutive panels, the fibre was perfused for 3–4 min with the relaxing solution (R). Arrows indicate solution changes. In all solutions, the pMg was 3·50 and the pMgATP was 2·50. The stability constant of Schwarzenbach *et al.* (1957) was used for the Ca-EGTA complex. All solutions except the caffeine solution (C) contained 4·0 mM total EGTA. The relaxing solution (R) was at pCa 9·00 and pH 7·00. The caffeine solution (C) was at pCa 8·30 and pH 7·00; it contained 0·20 mM total EGTA and 10 mM caffeine. The tonic tension observed during loading at pH 7·40 was caused by the effect of this pH on the sensitivity of the myofilaments to Ca^{2+}.

for loading the sarcoplasmic reticulum. The major finding was that the pH optimum for Ca^{2+} loading of the sarcoplasmic reticulum depended upon the [free Ca^{2+}]. Loading with a lower [free Ca^{2+}] resulted in a higher pH optimum (Table 2). Thus, the optimum for Ca^{2+} loading of the reticulum was between pH 7·00 and 7·40 in the presence of a pCa of 7·75 and between pH 6·60 and 6·20 at pCa 6·00. In addition, the amplitude of the contraction induced by caffeine at all the pH values studied increased when the [free Ca^{2+}] used for the loading was increased from pCa 7·75 to pCa 6·00.

TABLE 2. Amplitude of the caffeine-induced contraction after 2–3 min loading at various pCa and pH values. The amplitude of the caffeine-induced contraction developed by a given cell was expressed as a percentage of the tension developed by the same cell at pCa 5·00 and pH 7·00. Data are given in the following format: mean ± S.D. (sample size). In all cases, the loading was done in the presence of 4·0 mM total EGTA, pMg 3·50 and pMgATP 2·50. The values for the pCa were calculated with the equilibrium constants for Ca-EGTA defined by Schwarzenbach *et al.* (1957)

Skeletal muscle

pH	pCa 7·75	pCa 7·00	pCa 6·00
7·40	40·27 ± 8·21 (11)	48·28 ± 9·21 (11)	51·71 ± 8·49 (10)
7·00	38·11 ± 6·22 (12)	71·13 ± 4·83 (10)	76·11 ± 6·52 (9)
6·60	30·44 ± 4·13 (9)	63·64 ± 6·87 (12)	95·13 ± 3·02 (8)
6·20	20·88 ± 2·70 (8)	33·24 ± 3·10 (9)	92·14 ± 2·89 (7)

Cardiac muscle

pH	pCa 7·75	pCa 7·00	pCa 6·50
7·40	36·67 ± 7·11 (13)	20·15 ± 8·31 (11)	9·32 ± 4·21 (11)
7·00	30·10 ± 5·14 (11)	19·21 ± 6·24 (12)	7·14 ± 4·17 (13)
6·60	20·33 ± 5·21 (8)	18·35 ± 8·25 (9)	6·21 ± 2·82 (8)
6·20	14·67 ± 2·80 (10)	13·28 ± 7·80 (8)	8·32 ± 3·61 (7)

The same type of experiment was done in skinned cardiac cells (Fig. 4*A–D*). When the loading perfusion was at pCa 7·75, the amplitude of the caffeine-induced contraction was maximum for a loading pH between 7·00 and 7·40, or even higher than 7·40 since the amplitude of the contraction was significantly larger after loading at pH 7·40 than after loading at pH 7·00 (Table 2). Thus, the results obtained in cardiac and skeletal muscle cells after loading at pCa 7·75 were similar and the normalized values of the amplitude of the contractions differed little in the two tissues. For instance, there was no significant difference between the normalized amplitudes of the contractions induced by caffeine in the two tissues after loading at pH 7·40 and pCa 7·75. Similarly, the pH optimum was in the same range in the two tissues (Table 2).

In contrast, the results obtained from the two tissues were markedly different when the [free Ca^{2+}] was increased in the solution used for loading the sarcoplasmic reticulum (Table 2). In skinned cardiac cells, an increase of the [free Ca^{2+}] from pCa 7·75 to pCa 7·00 resulted in a decreasc, or at least in no increase, in the amplitude of the caffeine-induced contraction. These results were attributed to a Ca^{2+}-induced release of Ca^{2+} from the sarcoplasmic reticulum that started to occur when the [free Ca^{2+}] reached a pCa between 7·75 and 7·00. This was obvious at pH 7·40 and 7·00 because of the significant decrease of the amplitude of the caffeine-induced contraction when the [free Ca^{2+}] was increased from pCa 7·75 to pCa 7·00. An increase of the [free Ca^{2+}]

to pCa 6·50 was required, however, to decrease significantly the caffeine-induced contraction obtained after loading at pH 6·60 or 6·20. The finding that the amplitude of the caffeine-induced contraction was smaller after loading at pCa 6·50 than at pCa 7·00 in the presence of pH 7·00 or 7·40 was consistent with the hypothesis that the Ca^{2+}-induced release is not an all-or-none process but is graded with the [free Ca^{2+}] that triggers it (Fabiato & Fabiato, 1975*a*).

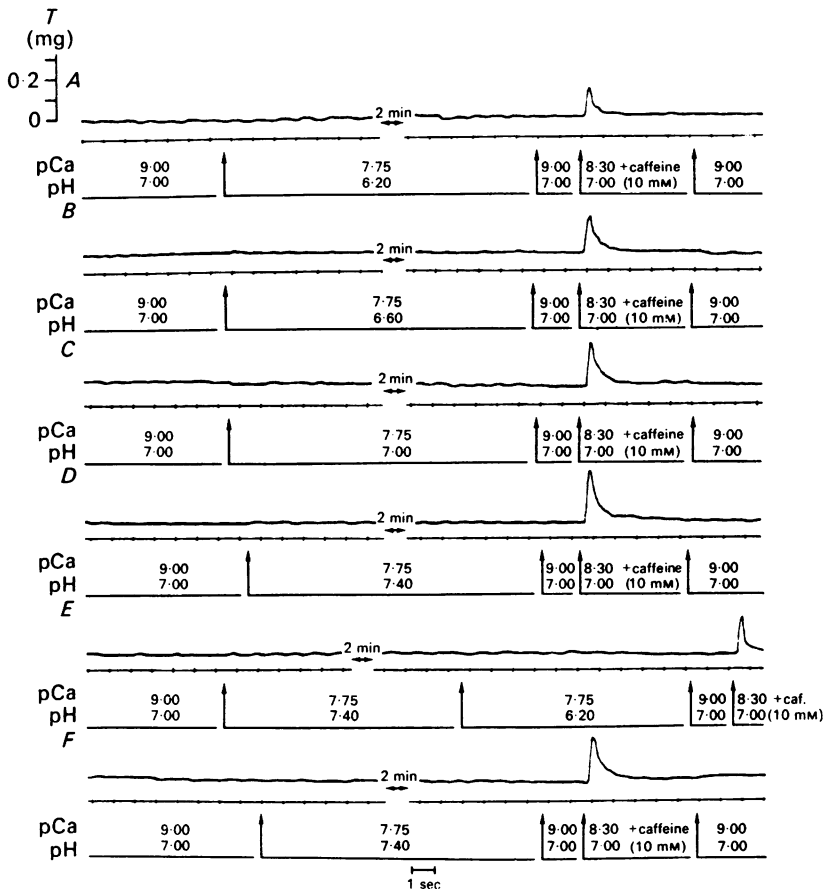

Fig. 4. Effects of changing the pH in the Ca^{2+}-loading solution with or without an additional brief exposure to another pH on the contraction induced by caffeine at pH 7·00 on an 11 μm wide and 49 μm long skinned cardiac cell from the rat ventricle. Arrows indicate solution changes. The experiments were done in the order in which they are shown. Between the recordings shown in two consecutive panels, the cell was perfused by the relaxing solution at pH 7·00 and pCa 9·00. The pMg was 3·50 and the pMgATP was 2·50. The [total EGTA] was 4·0 mM in all solutions except in the caffeine-containing solution, in which it was 0·20 mM. The stability constant of Schwarzenbach *et al.* (1957) was used for the Ca-EGTA complex. Note that the time scale is different from that used in Fig. 3.

The preceding data were obtained with a constant concentration of total EGTA for loading the sarcoplasmic reticulum. The same pCa was obtained by adding less total Ca at a lower pH. Thus, the buffer capacity was not constant but decreased

when the pH was lowered because the apparent stability constant for the Ca-EGTA complex decreased. It was necessary to eliminate the possibility that varying the Ca^{2+}-buffer capacity of the solution might, in itself, influence the Ca^{2+} loading of the sarcoplasmic reticulum. For instance, the latter might have competed more efficiently for Ca^{2+} binding with a weaker Ca-EGTA buffer. Additionally, a stronger Ca-EGTA buffer might have increased the membrane permeability of the sarcoplasmic reticulum

TABLE 3. Amplitude of the caffeine-induced contraction after 2–3 min loading in the presence of a constant concentration of Ca-EGTA buffer for the various pH values studied at each pCa. The [Ca-EGTA] used is indicated in parentheses below each pCa value. The data concerning the amplitude of the caffeine-induced contraction are given according to the same format as in Table 3. The [total EGTA] used at each pH to maintain the specified [Ca-EGTA] is indicated in parentheses below the corresponding data on the amplitude of the contraction. As for Table 2, the loading was done in the presence of a pMg 3·50 and pMgATP 2·50. The ionic strength was maintained at 0·160 M. The values for the pCa were calculated with the equilibrium constants for Ca-EGTA defined by Schwarzenbach *et al.* (1957)

Skeletal muscle

pH	pCa 7·75 (0·317 mM Ca-EGTA)	pCa 7·00 (1·305 mM Ca-EGTA)	pCa 6·00 (3·315 mM Ca-EGTA)
7·40	42·18 ± 9·11 (10) (0·935 mM total EGTA)	47·28 ± 8·17 (10) (1·76 mM total EGTA)	50·79 ± 9·32 (10) (3·43 mM total EGTA)
7·00	38·11 ± 6·22 (12) (4·00 mM total EGTA)	71·13 ± 4·83 (10) (4·00 mM total EGTA)	76·11 ± 6·52 (9) (4·00 mM total EGTA)
6·60	22·44 ± 5·18 (10) (23·52 mM total EGTA)	58·12 ± 2·37 (10) (18·28 mM total EGTA)	96·10 ± 4·35 (11) (7·63 mM total EGTA)

Cardiac muscle

pH	pCa 7·75 (0·317 mM Ca-EGTA)	pCa 7·00 (1·305 mM Ca-EGTA)	pCa 6·50 (2·419 mM Ca-EGTA)
7·40	39·67 ± 8·20 (12) (0·935 mM total EGTA)	22·14 ± 6·14 (10) (1·76 mM total EGTA)	8·75 ± 3·18 (10) (2·68 mM total EGTA)
7·00	30·10 ± 5·14 (11) (4·00 mM total EGTA)	19·21 ± 6·24 (12) (4·00 mM total EGTA)	7·14 ± 4·17 (13) (4·00 mM total EGTA)
6·60	15·27 ± 6·12 (9) (23·52 mM total EGTA)	14·12 ± 7·14 (9) (18·28 mM total EGTA)	5·12 ± 2·12 (9) (12·40 mM total EGTA)

(Duggan & Martonosi, 1970, obtained leaky reticulum vesicles by incubating fragmented sarcoplasmic reticulum in the presence of a high [total EGTA] at pH 9·00). Accordingly, control experiments were done in which the Ca^{2+}-buffer capacity measured by the total concentration of Ca-EGTA (all species) was kept constant for a given pCa at various pH values (Table 3). This required a variation of the [total EGTA] and was possible only for pH 7·40, 7·00 and 6·60 because the concentration of free EGTA required at pH 6·20 would not have been compatible with maintaining the ionic strength at 0·160 M. These experiments using a constant [Ca-EGTA] for Ca^{2+} loading confirmed the results obtained in the presence of a constant [total EGTA]. The ranges of optimum pH values for Ca^{2+} loading with solutions at various [free Ca^{2+}] values appeared to be the same whether [total EGTA] or [Ca-EGTA] was maintained constant (Tables 2 and 3). Yet, the significant difference between

the data obtained under the two conditions for the amplitude of the caffeine-induced contraction after loading at pH 6·60 and pCa 7·75 shows that these control experiments were necessary.

In summary, these Ca^{2+}-loading experiments permitted two conclusions. First, in skeletal muscle cells the pH optimum for loading the sarcoplasmic reticulum was more acidic when the [free Ca^{2+}] used for loading was higher. Secondly, the sarcoplasmic reticulum of cardiac muscle could not be loaded with a high [free Ca^{2+}] because this high [free Ca^{2+}] induced a Ca^{2+} release. Consequently, the pH optimum for loading the cardiac sarcoplasmic reticulum with Ca^{2+} was in the alkaline range.

A second type of experiments was done to see if, as shown by Nakamaru & Schwartz (1970, 1972) for the isolated sarcoplasmic reticulum, a sudden increase of pH during the loading period induced a Ca^{2+} release from the sarcoplasmic reticulum. In fact, just the opposite was observed when the initial loading solution was at pCa 7·75 in either skinned cardiac or skinned skeletal muscle fibres: a brief increase of pH increased the Ca^{2+} loading of the sarcoplasmic reticulum and, conversely, a brief decrease of pH decreased the loading. An example of such an experiment in a skinned cardiac cell is shown in Fig. 4*E* with controls in Fig. 4*D* and *F*. Only when the skeletal muscle sarcoplasmic reticulum had been loaded with a solution at pCa 6·00 did an increase of pH from 6·20 to 7·40 result in a decrease of Ca^{2+} loading. Yet, even under these conditions, the increase of pH from 6·20 to 7·40 did not produce a fast release of Ca^{2+} but a slow change in the Ca^{2+} loading of the sarcoplasmic reticulum. The effect of the second perfusion at pH 7·40 was not significant when its duration was less than 2 sec. The duration of the perfusion at pH 7·40 had to be increased to more than 30 sec in order to reduce caffeine-induced contraction to an amplitude equal to the mean of those obtained after 2 min loading at pH 6·20 and 7·40, respectively (data not shown).

The method using a high [total EGTA] to study the Ca^{2+} loading of and Ca^{2+} release from the sarcoplasmic reticulum places it under unphysiological conditions since there is obviously no high concentration of total EGTA in the intact cell. Experiments using a low [total EGTA] were done only in skinned cardiac cells. When the [total EGTA] was 0·050 mM, the total amount of Ca released from the tissue was sufficient to compete with the weak Ca-EGTA buffer. Then a small increase of the [free Ca^{2+}] resulted in the development of cyclic contractions in skinned cardiac cells (but not in skinned skeletal muscle fibres). These cyclic contractions were attributed to a Ca^{2+}-induced release of Ca^{2+} from the sarcoplasmic reticulum (Fabiato & Fabiato, 1975*a*), which could be a physiological mechanism of activation in the intact cardiac muscle (Fabiato & Fabiato, 1977).

Comparison of the effects of the four pH values studied on the cyclic contractions observed in the presence of 0·050 mM total EGTA required a [free Ca^{2+}] of pCa 6·50 in order to render it feasible to make the solution at pH 6·20. The variations in amplitude of the cyclic contractions at different pH values (Fig. 5) had no quantitative significance because both the sensitivity of the myofilaments to Ca^{2+} and the buffer capacity of 0·050 mM total EGTA varied when the pH was changed. Previous results have shown that the amplitude of the cyclic contractions decreases when the buffer capacity is increased (Fabiato & Fabiato, 1975*a*). Thus, the percentage of decrease in amplitude of the cyclic contractions produced by decreasing pH should

have been larger than shown in this experiment if the [Ca-EGTA] had been maintained constant (which was not technically possible for the complete pH range).

Despite the unphysiologically high [free Ca^{2+}], two significant observations were possible. First, a decrease of pH decreased, and an increase of pH increased, the frequency of the cyclic contractions. This suggests that acidosis decreases and alkalosis increases the rate of Ca^{2+} accumulation by the sarcoplasmic reticulum (see Fabiato & Fabiato, 1975*a* for rationale). Secondly, the changes in amplitude and

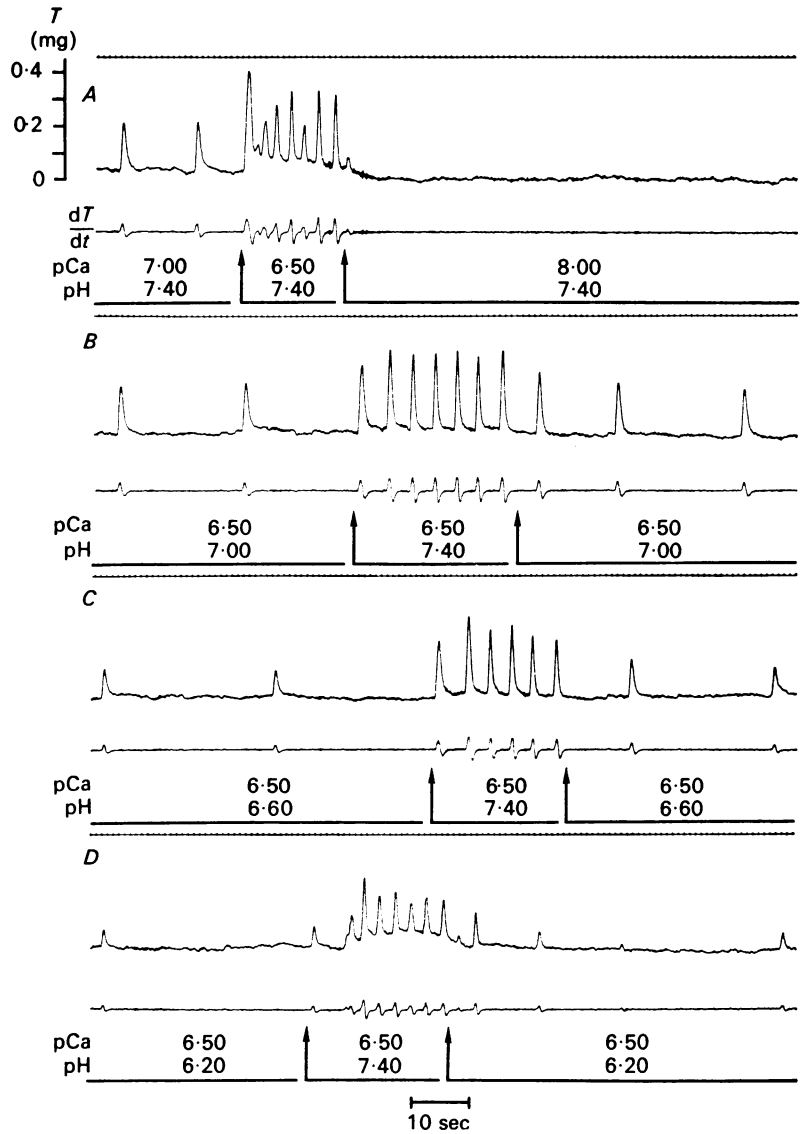

Fig. 5. Effect of varying pCa (panel *A*) or pH (panels *B*, *C* and *D*) on the phasic contractions obtained in a 10 μm wide skinned cardiac cell from the rat ventricle. Arrows indicate solution changes. In all solutions, the [total EGTA] was 0·050 mM, the pMg was 3·50 and the pMgATP was 2·50. The stability constant of Schwarzenbach *et al.* (1957) was used for the Ca-EGTA complex.

frequency of the cyclic contractions produced by a decrease or an increase of pH were slow, taking many seconds to be completed (Fig. 5*B–D*). These data are in sharp contrast to the sudden effects of varying pCa on the cyclic contractions (Fig. 5*A*). This again suggests that varying pH causes only slow changes in the Ca^{2+} transport by the sarcoplasmic reticulum.

The second conclusion implies that changes of intracellular pH are unlikely to be the physiological trigger for the Ca^{2+} release from the sarcoplasmic reticulum. But varying pH influenced the Ca^{2+}-induced release of Ca^{2+} from the sarcoplasmic reticulum. When the skinned cardiac cells had been equilibrated for 1 min at various pH values in the presence of a constant [Ca-EGTA], a lower pH resulted in a higher [free Ca^{2+}] threshold for Ca^{2+}-induced release of Ca^{2+} and in the release of a smaller amount of Ca^{2+} by a given [free Ca^{2+}] trigger. In the presence of 0·020 mM Ca-EGTA (obtained with a [total EGTA] of 0·050 mM at pH 7·40 and 0·110 mM at pH 7·00), the minimum [free Ca^{2+}] required for the induction of cyclic contractions was pCa 7·65 ± 0·03 (S.D. of eight observations) at pH 7·40 and pCa 7·35 ± 0·05 (S.D. of seven observations) at pH 7·00. An increase of the [free Ca^{2+}] requirement for Ca^{2+}-induced release of Ca^{2+} at a lower pH was already apparent in the data of Tables 2 and 3. To evaluate the influence of varying pH on the amount of Ca^{2+} released, the myoplasmic [free Ca^{2+}] reached during Ca^{2+} release was inferred from a comparison between the normalized amplitude of the cyclic contractions obtained at a given pH to the force–pCa curve obtained at the same pH (Fig. 1*B*). In the presence of 0·030 mM Ca-EGTA (obtained with a [total EGTA] of 0·034 mM at pH 7·40, 0·050 mM at pH 7·00 and 0·155 mM at pH 6·60), the amplitude of the cyclic contractions triggered by the same pCa of 6·50 corresponded to a myoplasmic pCa of 5·99 ± 0·04 (S.D. of five observations) at pH 7·40 and of 6·40 ± 0·11 (S.D. of six observations) at pH 7·00, whereas no cyclic concentrations were detectable at pH 6·60.

In summary, decreasing pH depressed not only the Ca^{2+} loading of the cardiac sarcoplasmic reticulum and the rate of Ca^{2+} accumulation (frequency of the cyclic contractions) but also the Ca^{2+}-induced release of Ca^{2+}. These and previous data (Fabiato & Fabiato, 1975*a*) suggest that the Ca^{2+}-induced release of Ca^{2+} is positively correlated with the Ca^{2+} accumulation by the sarcoplasmic reticulum. Therefore, the conclusion of Nakamaru & Schwartz (1970) that acidosis inhibits the Ca^{2+} release from the sarcoplasmic reticulum is applicable to the Ca^{2+}-induced release of Ca^{2+} precisely because the results obtained for Ca^{2+} loading in skinned cardiac cells are opposite to those observed by Nakamaru & Schwartz for oxalate-supported Ca^{2+} uptake by the isolated sarcoplasmic reticulum.

No study has been made of the effect of acidosis on the Ca^{2+} metabolism by the cardiac mitochondria because previous data have shown that the mitochondria function as a slow Ca^{2+} buffer only when the [free Ca^{2+}] is higher than pCa 6·50–6·00 (Fabiato & Fabiato, 1975*a*), which is largely above the diastolic myoplasmic [free Ca^{2+}]. They may become damaged by Ca^{2+} overload during acidosis because the depression of the Ca^{2+} accumulation by the sarcoplasmic reticulum increases the myoplasmic [free Ca^{2+}]. Then they may stop functioning as a Ca^{2+} buffer and this may result in the development of a resting tension (similar to that which occurred at the end of the experiment shown in Fig. 5). Their buffer capacity may also be reduced by a competition between H^+ and Ca^{2+} for mitochondrial binding sites

(Scarpa & Azzi, 1968). But the failure of the Ca^{2+} buffering by the mitochondria (equivalent to a slow Ca^{2+} release from the mitochondria) is a consequence rather than the cause of the depression of the excitation–contraction coupling process. Therefore, perturbations of Ca^{2+} metabolism by the mitochondria are unlikely to be a primary cause of the greater sensitivity of cardiac than skeletal muscle contraction to acidosis.

Finally, the effects of varying pH on phasic contractions induced in skinned skeletal muscle fibres by either Ca^{2+}-induced release of Ca^{2+} from the sarcoplasmic reticulum or by ionic modifications capable of depolarizing its membrane were not studied because neither mechanism seems likely to be representative of the physiological mechanism initiating the Ca^{2+} release in the intact skeletal muscle (Fabiato & Fabiato, 1977). (Ca^{2+}-induced release may amplify the Ca^{2+} release triggered by another unknown process, however.) Accordingly, in the subsequent discussion of the physiological significance of the data, we shall use the following working hypothesis: whatever may be the mechanism triggering Ca^{2+} release from the sarcoplasmic reticulum of the intact skeletal muscle, more Ca^{2+} is likely to be released from a more fully loaded sarcoplasmic reticulum.

DISCUSSION

Effects of pH on the myofilaments

The observation made in the present study that the effect of varying pH on the submaximal tension is more pronounced in an alkaline than in an acidic pH range is also apparent in some studies on the pH dependence of the myofibrillar ATPase activity (Williams *et al.* 1975). Even after normalization of the values of submaximal tension as a function of the maximum tension obtained at each pH, the force–pCa curves obtained from skinned cells of both cardiac and skeletal muscle do not suggest a simple competition between H^+ and Ca^{2+} for a single class of binding sites on troponin. Whether such a competition even exists has been recently questioned by Fuchs (1974), who challenged the previous data of Fuchs, Reddy & Briggs (1970). In addition, the troponin-tropomyosin system may not be the only Ca^{2+}-regulatory system in cardiac and skeletal muscles (Bailin, 1975). Finally, the development of force upon Ca^{2+} activation involves many possibly pH-dependent biochemical reactions other than Ca^{2+} binding to the regulatory proteins.

The more pronounced negative inotropic effect of acidosis in cardiac than in skeletal muscle cannot be explained by the depression of the maximum tension since this effect appeared, if anything, smaller in skinned cardiac cells than in skinned skeletal muscle fibres. In general, investigators who limited their studies to a pH range above 6·50 failed to show this depressive effect of acidosis on either maximum tension or myofibrillar ATPase activity (Bozler, 1968; Ashley & Moisescu, 1974; Williams *et al.* 1975; Kentish & Natler, 1977). Except for Chen-Liu & Endo (1973), all the authors who used a broad range of pH variations observed that acidosis depressed maximum tension or ATPase activity (Schädler, 1967; Portzehl *et al.* 1969; Robertson & Kerrick, 1976).

Therefore, the most significant contribution of these force–pCa curves to an explanation for the larger effect of acidosis in cardiac than in skeletal muscle is the

finding that decreasing pH depresses the submaximal tension more in the former tissue. Our comparison shows a smaller difference than that reported by Schädler (1967) but Schädler's studies were done in animal species different from those we used and with glycerinated fibres that may have an altered sensitivity to Ca^{2+} (Abbott, 1973).

Effects of pH on the sarcoplasmic reticulum

There is some evidence that the pH optimum for the Ca^{2+} transport by the fragmented sarcoplasmic reticulum may be slightly more alkaline in cardiac than in skeletal muscle (Shigekawa, Finegan & Katz, 1976). However, the values reported in the literature for the pH optimum for Ca^{2+} transport and binding by the isolated sarcoplasmic reticulum are spread between pH 6·00 and 8·30 (we apologize for not reviewing this literature here). The use of oxalate in most of these studies complicates the comparison of these data to those obtained from skinned muscle cells. Accordingly, the discussion of the significance of the ranges of pH optimum for Ca^{2+} loading of the sarcoplasmic reticulum of skinned cardiac and skeletal muscle cells will be limited to testing whether they can explain the observations made in intact muscle.

Very schematically, all the differential results reported in skinned cells from cardiac and skeletal muscles could be explained by a single difference: the Ca^{2+}-induced release of Ca^{2+} from the sarcoplasmic reticulum is obtained with a much lower myoplasmic [free Ca^{2+}] in cardiac than in skeletal muscle (Fabiato & Fabiato, 1977). Among other findings supporting this working hypothesis, it was observed that the effect of Mg^{2+} on the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles becomes identical when the Ca^{2+}-induced release of Ca^{2+} is enhanced in the latter tissue by caffeine (Fabiato & Fabiato, 1975*b*). Similarly, both the pH optimum for loading the sarcoplasmic reticulum and the relative amplitude of the caffeine-induced contraction are almost identical in the two preparations when they are equilibrated with an external pCa of 7·75. The differences appear when the external [free Ca^{2+}] is increased: this results in a loading of the sarcoplasmic reticulum of skeletal muscle but in a Ca^{2+}-induced release of Ca^{2+} that 'unloads' the cardiac reticulum. Then, the pH optimum for loading the skeletal muscle sarcoplasmic reticulum becomes more acidic and the relative amplitude of the caffeine-induced contraction becomes much larger than in cardiac muscle (close to 100% of the maximum tension). The problem in applying these data to the intact muscles consists of defining which loading pCa corresponds to a physiological loading of the sarcoplasmic reticulum in both tissues.

The condition likely to be representative of the physiology of the resting cardiac cell is a loading that does not induce a Ca^{2+} release from the sarcoplasmic reticulum. This is represented by a perfusion of skinned cardiac cells at pCa 7·75 for which the pH optimum for the Ca^{2+} loading of the sarcoplasmic reticulum is around 7·40. If this value is correct, an intracellular acidosis should always impair Ca^{2+} accumulation by the sarcoplasmic reticulum in the intact cardiac muscle. This should result in a decrease of the rate of relaxation. Indeed, a decrease of the relaxation rate was observed during the intracellular acidosis produced by CO_2 (Pannier & Leusen, 1968; Poole-Wilson & Langer, 1975), by hypoxia (Tyberg, Yeatman, Parmley,

Urschel & Sonnenblick, 1970) and by ischaemia (Weisfeldt, Armstrong, Scully, Sanders & Daggett, 1974). Lowering pH also depresses the Ca^{2+}-induced release of Ca^{2+} from the sarcoplasmic reticulum. Thus, depression by acidosis of the sarcoplasmic reticulum function could be, at least, a part of the mechanism for the decrease in both contraction amplitude and relaxation rate observed during acute myocardial infarction (Weisfeldt *et al.* 1974).

For the skeletal muscle cells, the physiologically representative condition would be one for which no tension is developed by the myofilaments before the Ca^{2+} release from the sarcoplasmic reticulum. The threshold for contraction in skinned skeletal muscle fibres corresponds to a [free Ca^{2+}] higher than pCa 6·75 at pH 7·00, which may be about the physiological intracellular pH (Waddell & Bates, 1969). Thus, the sarcoplasmic reticulum of the intact skeletal muscle could be loaded as much as it is in skinned fibres by a perfusion at this pCa 6·75. Taking a less optimal condition represented by a perfusion at pCa 7·00, it appears that the pH optimum would be between 7·00 and 6·60. If this pH range is correct, a small decrease of intracellular pH should modify little the contraction since the effect of pH on the sarcoplasmic reticulum may compensate for its effects on the myofilaments. A predominant effect on the sarcoplasmic reticulum of a small acidosis may even explain the observation of Pannier *et al.* (1970) that an increase of P_{CO_2} paradoxically enhanced the contraction developed by the rat soleus. In contrast, an acidosis lowering the pH to between 6·60 and 6·20 should impair the function of the sarcoplasmic reticulum. González-Serratos, Borrero & Franzini-Armstrong (1974) indeed provided evidence that a decrease of 0·70 units of the intracellular pH in frog skeletal muscle resulted in a depression of the Ca^{2+} transport by the sarcoplasmic reticulum.

Therefore, some observations in intact muscles provide circumstantial evidence that the ranges of pH optimum for Ca^{2+} loading of the sarcoplasmic reticulum defined in skinned muscle cells may be relevant to the physiology of the intact cells. Obviously, differences may exist between different types of cardiac or skeletal muscles and among various animal species. The aim of the present study was not to define very specific values for the pH optima but to propose a mechanism for the different effects of acidosis in skeletal and mammalian cardiac muscles.

Causes for the more pronounced negative inotropic effect of acidosis in cardiac than in skeletal muscle

In conclusion, the data obtained from skinned muscle cells suggest that the action of acidosis on the sarcoplasmic reticulum always accentuates its depressive effects on the myofilaments in cardiac muscle, whereas it may minimize them in skeletal muscle. First, the amount of Ca^{2+} released from the sarcoplasmic reticulum of cardiac muscle is reduced by even a moderate acidosis. Secondly, this amount is always much less than needed to activate the myofilaments completely. Consequently, the Ca^{2+} released from the sarcoplasmic reticulum will produce a low level of activation, for which the effects of acidosis are much more pronounced than they would be for maximal activation.

In contrast, a small acidosis increases the Ca^{2+} content of the sarcoplasmic reticulum of skeletal muscle and the larger amount of Ca^{2+} released will compensate for

any decrease in the sensitivity of the myofilaments to the Ca^{2+}. Even if a larger acidosis does decrease the Ca^{2+} content of the sarcoplasmic reticulum, this amount may still be sufficient to saturate the regulatory protein(s). Then, the only effect observed would be that of the pH variation on the maximum tension developed by the myofilaments, which is a decrease of tension of only 30 % when the intracellular pH is as low as 6·20.

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